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Lipase-catalysed resolution of *N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide Synthesis of (*R*)-GABOB and (*R*)-carnitine

Ahmed Kamal *, Tadiparthi Krishnaji, M. Naseer A. Khan

Biotransformation Laboratory, Division of Organic Chemistry, Indian Institute of Chemical Technology, Hyderabad 500007, India

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Abstract

The lipase-mediated resolution of *N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide using various lipases in different solvents has been studied and this intermediate was utilized towards the synthesis of enantiomerically enriched (*R*)-GABOB and (*R*)-carnitine in high yields.

Keywords: Synthesis; Enzymes; Kinetic resolution; (*R*)-GABOB; (*R*)-carnitine

1. Introduction

γ-Amino-β-hydroxybutyric acid (GABOB) is a compound of great pharmacological importance because of its biological function as a neuromodulator in the mammalian central nervous system [1–4]. The (*R*)-form of GABOB is shown to have greater biological activity than its *S*-enantiomer [5]. Moreover, the (*R*)-isomer of GABOB serves as a precursor for (*R*)-carnitine, a compound having high level of medical significance. Carnitine is a vitamin like substance and plays an important role in converting stored body fat into energy. Its primary physiological function is to transport long chain fatty acids through the mitochondrial membrane [6] into the cellular compartments for oxidation where these fats can be converted into energy. Moreover, there are several medical indications for which carnitine has been prescribed [7,8], these include its usefulness as a pharmaceutical for hemodialysis [9] (hypolipidemic agent), heart diseases [7], etc.

The high biological importance associated with (*R*)-GABOB and (*R*)-carnitine continues to stimulate interest in many research groups [10] for the development of facile, efficient and economic approaches for their preparation (Fig. 1). Currently the majority of the world demand of (*R*)-carnitine (several thousand tonnes/year) is produced by biotransformation using a microorganism [10d–f].

2. Experimental

Reactions involving moisture sensitive reagents were performed under an inert atmosphere of nitrogen in glassware, which had been oven dried. Melting points were recorded on an electrothermal melting point apparatus and are uncorrected. Infrared spectra were recorded on Perkin-Elmer model 683 or 1310 spectrometers and are reported in wave numbers (cm^{-1}). ^1H NMR spectra were recorded as solutions in $CDCl_3$ and chemical shifts reported in parts per million (ppm, δ) on a Gemini 200 MHz, AV 300 MHz, instrument using tetramethylsilane (TMS) as an internal standard. Spectral patterns unless specified all solvents and reagents were of reagent grade and used without further purification. Spectral patterns are designated as s, singlet; d, doublet; dd, double doublet; t, triplet; br, broad; m, multiplet. Coupling constants are reported in hertz (Hz). Low-resolution mass spectra were recorded on CEC-21-100B Finnigan Mat 1210 or VG 7070H Micromass mass spectrometers. Analytical TLC of all reactions was performed on Merck prepared plates (silica gel 60F-254 on glass). Column chromatography was performed using Acme silica gel (100–200 mesh). Percentage yields are given for compounds. HPLC analysis was performed on an instrument that consisted of a Shimadzu LC-

* Corresponding author. Tel.: +91 40 27193157; fax: +91 40 27193189.
E-mail addresses: ahmedkamal@iict.res.in, ahmedkamal@iict.net.org (A. Kamal).

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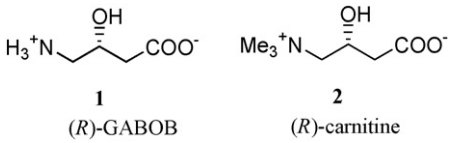


1 (*R*)-GABOB 2 (*R*)-carnitine

Fig. 1. Structures of (*R*)-GABOB and (*R*)-carnitine.

10AT system controller with a SPD-10A fixed wavelength UV monitor as detector. Optical rotations were measured on SEPA-300 (Horiba) digital polarimeter. Enantiomeric excess has been determined by chiral HPLC (chiral column ODH; Daicel).

2.1. Synthesis of 2-oxiranylmethyl-isoindole-1, 3-dione (*5*)

To a stirred solution of potassium pthalimide **3** (5.0 g, 27.02 mmol) in dry DMF (50 mL), epichlorohydrine **4** (3.74 g, 40.54 mmol) was added and heated under reflux conditions for 6 h. After completion of the reaction, crushed ice was added to the crude mixture, extracted with ether (3 × 100 mL) and dried over anhydrous Na_2SO_4. Purified by column chromatography using EtOAc:hexane (15:85) as eluent to give the epoxide **5** as a solid (4.4 g, 80%); m.p. 80–84 °C; FT IR (Neat): ν_{max} = 1770, 1712, 1393, 1059, 720 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.90–7.82 (m, 2H), 7.76–7.67 (m, 2H), 3.97 (dd, 1H, $J_1 = J_2 = 4.5$ Hz), 3.71 (dd, 1H, $J_1 = J_2 = 5.28$ Hz), 3.22–3.15 (m, 1H), 2.77 (t, 1H, $J = 4.5$ Hz), 2.68–2.64 (m, 1H); LCMS: 225.9 $(M + Na)^+$.

2.2. Synthesis of N-(3-cyano-2-hydroxy propan-1-yl)phthalimide (*6*)

To a stirred solution of epoxide **5** (2.0 g, 10.0 mmol) in EtOH (25 mL), NaCN (0.74 g, 15 mmol) and water (30 mL) were added for 10 h. After completion of the reaction, water was added to the crude reaction mixture, extracted with ethyl acetate (2 × 100 mL) and dried over anhydrous Na_2SO_4. Purified by column chromatography using EtOAc:hexane (1:1) as eluent to give the β-hydroxy nitrile **6** as a solid (1.93 g, 85%); m.p. 112–114 °C; FT IR (Neat): ν_{max} = 3478, 2932, 2245, 1713, 1688, 1403, 1014, 715 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.92–7.85 (m, 2H), 7.81–7.73 (m, 2H), 4.33–4.22 (m, 1H), 3.92 (d, $J = 5.3$ Hz, 2H), 3.30 (bs, 1H), 2.73–2.53 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 24.0, 43.0, 66.23, 75.0, 116.9, 123.6, 131.6, 134.4, 168.7; LCMS: 231 $(M + 1)^+$, 253 $(M + Na)^+$.

2.3. General procedure for the kinetic resolution of N-(3-cyano-2-hydroxy propan-1-yl)phthalimide (*6*)

To the compound **6** (0.5 g, 2.17 mmol) was dissolved in toluene–chloroform (30 mL, 8:2), lipase (0.5 g, w/w) and vinyl acetate (1.12 g, 13.04 mmol) were added successively in an orbital shaker at 30 °C. After about to 50% completion of the reaction as indicated by HPLC, the reaction mixture was filtered and washed with EtOAc. The two enantiomers were separated by column chromatography using EtOAc:hexane (1:1) as eluent afforded (*R*)-acetate-(*R*)-**7a** followed by unreacted (*S*)-alcohol-(*S*)-**6b**. Enantiomeric excess of (*R*)-acetate-(*R*)-**7a** and (*S*)-alcohol-(*S*)-**6b** have been determined by chiral HPLC (chiral column ODH; Daicel) employing hexane-isopropanol (80:20) as a mobile phase at 0.75 mL/min and monitored at UV (254 nm). Retention times for (*R*)-acetate (*R*)-**7a** and (*S*)-alcohol (*S*)-**6b** are 42.41 and 29.45 min, respectively.

2.4. N-(3-cyano-2-hydroxy propan-1-yl)phthalimide *6a*

$[\alpha]_D^{28} = +13.5$ (*c* 1.0, $CHCl_3$).

2.5. N-(3-cyano-2-hydroxy propan-1-yl)phthalimide *6b*

$[\alpha]_D^{28} = -10.5$ (*c* 1.0, $CHCl_3$).

2.6. Synthesis of N-(3-cyano-2-acetoxy propan-1-yl)phthalimide (*7*)

To a stirred solution of β-hydroxy nitrile 6 (0.5 g, 2.2 mmol) in CH_2Cl_2, acetic anhydride (0.45 g, 4.4 mmol), triethyl amine (0.44 g, 4.4 mmol) and DMAP (cat) were added successively at 0 °C for 1 h. After completion of the reaction, CH_2Cl_2 was added, washed with 5% HCl, brine, $NaHCO_3$ and dried over anhydrous Na_2SO_4. Purified by column chromatography using EtOAc:hexane (30:70) as eluent to give the β-acetoxy nitrile **7** as a solid (0.56, 95%); m.p. 114–117 °C; FT IR (Neat): ν_{max} = 2247, 1743, 1713, 1396, 1223, 1039 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.86–7.64 (m, 4H), 5.31–5.15 (m, 1H), 4.06–3.78 (m, 2H), 2.85–2.52 (m, 2H), 2.02 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.33, 168.01, 134.32, 131.7, 123.7, 115.73, 66.86, 39.72, 21.24, 20.72; LCMS: 295 $(M + Na)^+$.

2.7. N-(3-cyano-2-acetoxy propan-1-yl)phthalimide *7a*

$[\alpha]_D^{28} = +25.43$ (*c* 2.9, $CHCl_3$).

2.8. Synthesis of (R)-4-Amino-3-hydroxybutanoic acid (R)-GABOB (R)-*1*

The hydroxynitrile **6b** (1.0 g, 4.35 mmol) was heated at 95 °C with concentrated HCl (10 mL) for 10 h. The aqueous layer was washed with $CHCl_3$ (4 × 25 mL) and evaporated. The resulting crystalline product was dried under vacuum to give (*R*)-GABOB hydrochloride (0.37 g, 80%). The above hydrochloride was dissolved in distilled water (1 mL) and passed through a column containing Dowex 50 W × 2–100 resin (6 g) followed by distilled water (60 mL). The column was eluted with 25% aqueous ammonia (20 mL) and the ammonical eluent was evaporated to dryness under vacuum to provide (*R*)-GABOB (80%). Recrystalization of the (*R*)-GABOB from water–ethanol provided pure (*R*)-GABOB-**1** as white crystals in 70% yield; m.p. 211–213 °C; $[\alpha]_D^{28} = -20.7$ (*c* 1.0, H_2O); ^1H NMR (200 MHz, D_2O): δ 2.43 (d, 2H, $J = 5.9$ Hz), 2.95 (dd, 1H, $J_1 = 9.66$ Hz, $J_2 = 13.38$ Hz), 3.18 (dd, 1H, $J_1 = 3.72$ Hz, $J_2 = 13.38$ Hz), 4.10–4.30 (m, 1H); ^{13}C NMR (50 MHz, D_2O): δ 42.3, 44.0, 65.5, 178.5; mass (EI): 118 $(M^+ - H)$, 74, 60, 43.

Table 1
Transesterification of *N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide (**6**) with various lipases in toluene

Entry	Lipase[a]	Time (h)	Alcohol		Acetate		Conversion *c* (%)	*E*
			Yield[b] (%)	ee[c] (%)	Yield[b] (%)	ee[c] (%)		
1	PS-C	70	44	>99	46	>99	50	1055
2	PS-D	100	50	90	40	>99	48	624
3	PS	120	60	82	30	95	46	98
4	AK	168	44	90	47	90	50	58.38
5	P	168	80	15	12	98	13.2	10.12
6	Lipozyme	168	88	06	08	89	6.3	18.36
7	AYS	168	69	13	23	54	19	3.76
8	CRL	168	68	14	25	51	22	2.24
9	CAL-B	168	90	03	02	23	11.5	1.64

[a] *Pseudomonas cepacia* lipase immobilized on diatomite (PS-D), *Pseudomonas cepacia* (PS), *Pseudomonas cepacia* lipase immobilized on modified ceramic particals (PS-C), *Pseudomonas fluorescens* lipase (AK) are obtained from Amano Pharmaceutical company, Japan, *Pseudomonas fluorescens* lipase immobilized in sol–gel-AK on sintered glass (P), lipase immobilized from Mucor meihei (Lipozyme), *Candida antartica* lipase immobilized in sol–gel-AK on sintered glass (CAL B) are from Fluka, *Candida rugosa* lipase (CRL) (Sigma).
[b] Isolated yields.
[c] Determined by chiral HPLC (chiral column ODH; Daicel) employing hexane–isopropanol (80:20) as mobile phase at 0.75 mL/min and monitored by UV (254 nm).

2.9. Synthesis of (*R*)-carnitine (*R*)-**2**

Methylation of (*R*)-GABOB-**1** (0.5 g, 4.8 mmol) was carried out by reported method to furnish (*R*)-carnitine-**2** (0.42 g; 60% yield); m.p. 140–142 °C; $[\alpha]_D^{25} = -20.5$ (*c* 1.0, H_2O) {lit.[10a] m.p. 144–146 °C; $[\alpha]_D^{25} = -23.9$ (*c* 0.86, H_2O)}; ^{1}H NMR (200 MHz, D_2O): δ 2.60–2.70 (m, 2H), 3.24 (s, 9H), 3.48–3.55 (m, 2H), 4.60–4.75 (m, 1H); ^{13}C (D_2O): δ 5.8, 56.9, 66.9, 73.0, 180.9.

3. Results and discussion

In continuation of our earlier efforts [11] towards the preparation of biologically important compounds or their intermediates by using enzymes, we herein wish to report an efficient synthesis (*R*)-GABOB and (*R*)-carnitine. β-hydroxynitriles have importance both as reagents and as technical products in organic chemistry and have been extensively investigated and employed in synthetic organic chemistry for the preparation of intermediates of many naturally occurring bioactive compounds. Therefore, (*R*)-GABOB has been synthesized using optically pure *N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide.

Accordingly, epoxide **5** was prepared from potassium phthalimide **3** with epichlorohydrin **4** in DMF under reflux conditions in quantitave yield. Epoxide **5** was then treated with NaCN in ethanol gave β-hydroxy nitrile **6**, which was resolved enzymatically using different lipases (Table 1). Among all the lipases screened, lipase from *Pseudomonas cepacia* immobilized on modified ceramic particles (PS-C) also known as *Burkholderia cepacia* gave good conversion and high enantiomeric excess. Furthermore, the effect of solvents has also been investigated and it was observed that the transesterification in hydrophobic solvents such as toluene, diisopropyl ether, and hexane not only provided good yields, but also high enantiomeric excess for both (*S*)-alcohol and (*R*)-acetate. However, the hydrophilic solvents

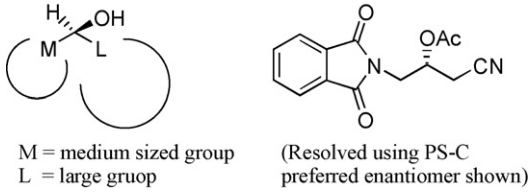
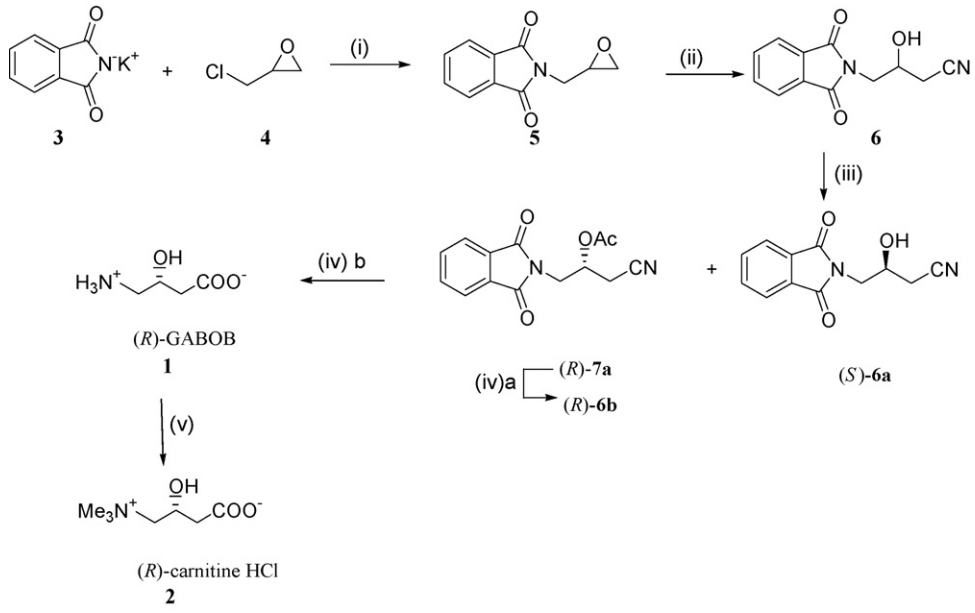


Fig. 2. Preferred enaniomer of **6** using lipase PS-C.

Table 2
Effect of solvents on the transesterification of *N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide (**6**) by lipase PS-C

Entry	Solvent	log *P*	Time (h)	Alcohol		Acetate		Conversion, *c* (%)	*E*
				Yield[a] (%)	ee[b] (%)	Yield[a] (%)	ee[b] (%)		
1	Toluene	2.5	70	44	>99	46	>99	50	1055
2	Diisopropylether	1.9	168	65	70	14	95	42	80.55
3	Hexane	3.5	168	48	83	44	86	49	33.18
4	Diethyl ether	–	168	40	99	53	84	54	59
5	Chloroform	2.0	168	54	69	38	95	42	857
6	Acetone	−0.23	168	86	11	07	>99	10	222
7	Tetrahydrofuran	0.49	168	90	04	03	>99	4	21
8	Dioxane	−1.1	168	91	04	03	>99	4	21

[a] Isolated yields.
[b] Determined by chiral HPLC (chiral column ODH; Daicel) employing hexane-isopropanol (80:20) as mobile phase at 0.75 mL/min and monitored by UV (254 nm).



Scheme 1. Reagents and conditions: (i) DMF, reflux, 6 h (80%); (ii) NaCN, EtOH-H_2O, 10 h (85%); (iii) Lipase, vinyl acetate, toluene/chloroform (8:2); (iv) (a) aqueous NH3 (25%), MeOH, 0 °C to rt (90%); (b) Concentrated HCl, reflux 10 h (88%); (v) MeI (80%).

such as THF or dioxane gave low yields, particularly the (*R*)-acetate and as a result the enantiomeric excess of (*S*)-alcohol was also greatly reduced. Therefore, toluene appears to be the solvent of choice for this type of a transesterification with regard to yields and enantiomeric excess (Table 2).

Alcohol **6** upon enzymatic resolution gave alcohol **6a** in >99% and acetate **7a** in >99%, respectively ($E = 1055$). The stereochemical preference of PS-C during kinetic resolution was in line with Kazlauskas' rule (Fig. 2) [12]. These two enantiomers were separated by column chromatography. The acetate **7a** was hydrolyzed using aqueous NH_3 (25%) in methanol gave alcohol **6b**. Further, alcohol **6b** was hydrolyzed using concentrated HCl under reflux conditions gave (*R*)-GABOB (*R*)-**1**, which on methylation gave (*R*)-carnitine (*R*)-**2** (Scheme 1).

4. Conclusion

In summary, an efficient method for the preparation of (±)-*N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide and its successful enzymatic resolution have been described. This lipase-mediated transesterification process has been optimized with respect to different lipases and solvents. Furthermore, enantiomerically pure *N*-(3-cyano-2-hydroxy propan-1-yl)phthalimide has been employed in the preparation of (*R*)-GABOB and (*R*)-carnitine.

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